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Can Ayahuasca and sleep loss change sexual performance in male rats?

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ABSTRACT

The ingestion of the beverage *Ayahuasca* usually occurs in religious ceremonies that are performed during the night leading to sleep deprivation. The purpose of the present study was to characterize the acute effects of *Ayahuasca* upon the sexual response of sleep deprived male rats. One group of sexually experienced male Wistar rats were submitted to a paradoxical sleep deprivation (PSD) protocol for 96 h, while another group spent the same amount of time in the home cage (CTRL). After this period, either saline or *Ayahuasca* drink (250, 500 and 1000 $\mu g\,mL^{-1}$) was administered by gavage and sexual behavior and hormonal concentrations were measured. *Ayahuasca* alone significantly decreased sexual performance at all doses. However, in sleep deprived rats, the lower dose increased sexual performance while the intermediate dose produced a detrimental effect on sexual response compared to the CTRL rats at the same dose. Regarding the hormonal analyses, a lower testosterone concentration was observed in sleep-deprived saline rats in relation to the CTRL group. Progesterone was significantly lower only in PSD rats at the dose 500 $\mu g\,mL^{-1}$ compared with CTRL-500 $\mu g\,mL^{-1}$ group. Corticosterone was unchanged among the groups evaluated. Our results suggest that *Ayahuasca* intake markedly impaired sexual performance alone, but, when combined with sleep deprivation, had significant, but heterogeneous, effects on male sexual response.

1. Introduction

The beverage *Ayahuasca* has been used ritualistically from time immemorial by over 70 tribes of native peoples of South America, across Brazil, Peru, Bolivia, Colombia, Ecuador, and Venezuela. The ingestion of the beverage traditionally occurs within a strong religious context. No longer exclusive to native peoples, *Ayahuasca* is consumed in big cities in a different way from how it was among its original consumers; while only a small percentage of tribes' people consume it during rituals, all members of urban religious sects like "Santo Daime" and other agnostic groups use ingestion of *Ayahuasca* as the central event within a ceremonial context (Callaway et al., 1999).

Ayahuasca is composed by decoction of two plants: *Banisteriopsis caapi* and *Psychotria viridis*. The woody vines of *B. caapi* contain some types of β-carbolines: harmine, harmaline and tetrahydroharmine, which inhibit the monoamine oxidase enzyme (MAO). The leaves of *P. viridis* promote hallucinations in users due to the presence of *N*,*N*-dimethyltryptamine (DMT). The compound DMT is an indole alkaloid, structurally related to the neurotransmitter serotonin (Callaway and Grob, 1998; Riba et al., 2001). DMT has high affinity for 5-HT_{2A} and 5-HT_{2C} receptors in the brain and moderate to low affinity for the 5-HT_{1A} receptor (Keiser et al., 2009).

Under normal conditions, the DMT is rapidly metabolized by MAO endogenous (present in the liver and gut), but as this is inhibited by β-carbolines (harmine, harmaline and tetrahydroharmine), the degradation of DMT does not occur and it can act freely in the central nervous system, enabling users to experience a wide range of hallucinations (Callaway and Grob, 1998). The synergistic interaction of these alkaloids is the basis of the psychotropic action of *Ayahuasca* (McKenna, 2004). The chemical structures of main alkaloids presents in *Ayahuasca* extract and the serotonin are displayed in Fig. 1.

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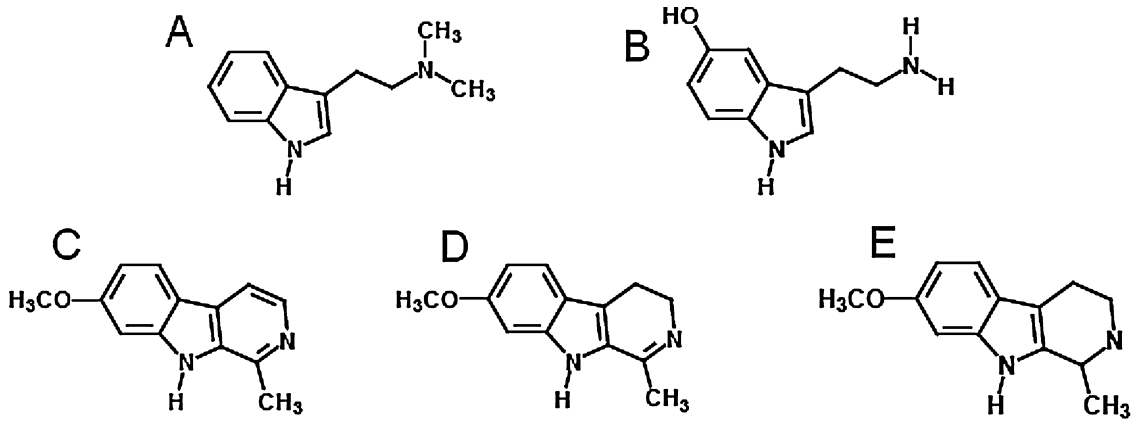

Fig. 1. Chemical structure of main alkaloids in *Ayahuasca* extract and Serotonin structure. Legends: (A) dimethyltriptamine; (B) serotonin (5-hydroxitriptamine); (C) harmine; (D) harmaline; (E) tetrahydroharmine.

There are no conclusive studies about the toxicity, safety, and possible adverse effects of *Ayahuasca*, but the ingestion of an average dose (usually 150 mL) leads to hallucination and emotional, perceptive, and memory alterations (Callaway and Grob, 1998; Shanon, 2003; Gable, 2007). Nausea, vomiting, and trembling have also been observed (Callaway and Grob, 1998). Hallucinogenic substances are routinely ingested nocturnally at parties and dance clubs on occasions often associated with sleep deprivation. In particular, *Ayahuasca* followers are subjected to sleep deprivation as the rituals extend for many hours during the night. Although the Santo Daime cult rituals which involve *Ayahuasca* begin at 22:00 h and last until 03:00 h, the hallucinogenic beverage is served only in the final parts of the ritual. Recently, clinical studies assessed neuropsychological, behavioral, neuroendocrine and immunological responses in subjects who ingested the *Ayahuasca* extract (Bouso et al., 2012, 2013; Dos Santos et al., 2011; Thomas et al., 2013).

Shortage of sleep alone can directly compromise an individual's quality of life, impacting, for instance, the immune system (Ruiz et al., 2012) and causing attention and memory deficits (Kumar and Jha, 2012). In addition, sexual behavior is altered by sleep loss. Our group has demonstrated that the reduction of sleep time induced by paradoxical sleep deprivation (PSD) may lead to an increase in motivational sexual responses, erections, and spontaneous ejaculations in male rats (Andersen et al., 2004, 2005, 2007), but decreases in sexual performance, observable in increased latency and reduced number of intromissions (Alvarenga et al., 2009).

It is known that the abusive ingestion of alcohol and cocaine may cause metabolic imbalances, leading to erectile dysfunction (Cocores et al., 1988). This fact suggests that sexual response can be modulated by substances of abuse. Moreover, the combination of sleep deprivation and drug abuse is known to induce genetic and neurochemical damage (Martins et al., 2008; Alvarenga et al., 2011). Given the religious and nocturnal nature of *Ayahuasca* use, it appears likely that it is ingested under conditions of sleep deprivation. The synergic action resulting from the association of sleep deprivation and drug abuse may aggravate the detrimental physical effects of sleep deprivation alone.

The Ayahuasca is a drug that can alter the central nervous system and thereby bring several consequences for our body. However, as it has a religious aspect and it is not much explored and few studies have shown the possible detrimental effects. Thus, given the historical association of drugs of abuse with lack of sleep and effects on sexual behavior, we proposed to examine the effects of Ayahuasca alone or in combination with sleep deprivation, upon male sexual function in rats, and the hormones involved in sexual function and stress. By doing this, we expect to clarify if this substance can promote marked physiological changes to our body, regardless of social, cultural or religious aspects.

2. Material and methods

2.1. Animals

Adult male Wistar rats were bred and raised in the animal facility of the Centro de Desenvolvimento de Modelos Experimentais para Medicina e Biologia (CEDEME) of the Universidade Federal de São Paulo. The animals were housed in group in a colony with a constant temperature of 22 ± 1 °C, a 12:12 h light-dark cycle (lights on at 07:00 h) and free access to water and food. All animals were treated in accordance with ethical guidelines (Andersen and Tufik, 2010) and NIH Publications no. 80-23 revised 1996 and all procedures were approved by the University's Ethics Committee (CEP no. 12/0302).

2.2. Training and sexual behavior evaluation

Initially, the rats were trained to acquire sexual experience. This protocol consists of exposing the male rat to a receptive female during 5 alternate days, which allowed standardization for the same degree of copulatory activity (Alvarenga et al., 2010). The rats that did not display any sexual response were excluded from the study. 24 h after the last training session, the rats with excellent sexual performance were selected and subjected to PSD for 96 h. Sexual behavior was evaluated immediately following this period.

Training and testing of sexual behavior was performed using a Plexiglas cylinder arena with a 45 cm diameter. Dim red lights shone during the dark phase of the light/dark cycle. A male was introduced into the arena 5 min before a female. Sexual receptivity in the female rats was established by administering estradiol benzoate (10 μg 0.1 mL^{-1} of sesame oil, sc, Sigma Chemical Co., St. Louis MO, USA) 48 h and 24 h prior to the test, and followed by administration of progesterone 4 h prior (500 μg 0.1 mL^{-1} of sesame oil, sc, Sigma Chemical Co., St. Louis MO, USA). Each test of sexual behavior lasted for 30 min after the introduction of the female, during which the following variables were recorded by a trained observer in the room: the first mount, intromission and ejaculation latencies, number of mounts (mounts with pelvic thrusting) and intromissions (mounts with pelvic thrusting and penile insertion) preceding the first ejaculation, total number of mounts, intromissions and ejaculations.

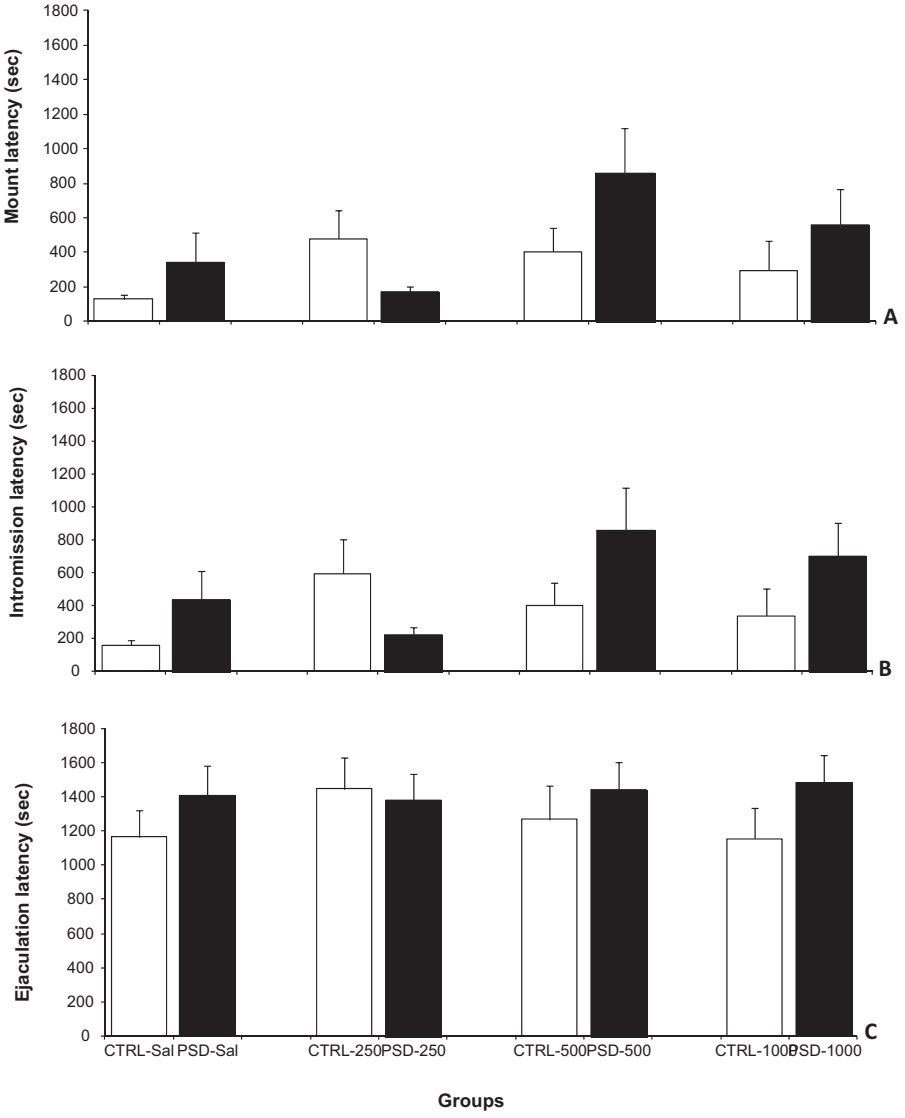

Fig. 2. Effect of *Ayahuasca* intake and/or paradoxical sleep deprivation (PSD) on latencies to mount (panel A), intromission (panel B) and ejaculation (panel C). Latencies are expressed as mean ± SEM (n = 10/groups).

2.3. Paradoxical sleep deprivation (PSD)

The experimental groups were subjected to 96 h of PSD using the modified multiple platform method. The 96 h duration for PSD was chosen because previous studies have demonstrated that the most dramatic alterations in behavior (Andersen et al., 2003) and hormone concentrations (Andersen et al., 2005) occur using this period of PSD. A total of 10 rats were placed, one at a time, inside a tiled water tank (143 cm × 41 cm × 30 cm) containing 14 circular platforms (each 6.5 cm in diameter) with the water level within 1 cm of the upper surface. The rats could move within the tank by jumping from one platform to another. When they reached the paradoxical phase of sleep, muscle atonia caused them to fall into the water and awaken. Throughout the study, the experimental room was maintained at a controlled temperature (22 ± 1 °C) with a 12 h light/dark cycle (lights on at 07:00 h and off at 19:00 h). The rats had free access to food and water located on a grid on top of the tank. The water in the tank was changed daily during the PSD period. All animals were submitted to the PSD period, and trained and tested in relation to sexual behavior at the same time, during the dark phase of the light-dark cycle (19:00 h).

2.4. Protocol designs

The animals that displayed excellent performance during sexual training (i.e.: animals showing a frequency of ejaculation between 70 and 100% on all training days to acquire sexual experience; for details in Alvarenga et al. (2010)) were randomly allocated into 8 independent groups (n = 10/group). The experimental groups underwent the PSD protocol for 96 h and were given *Ayahuasca* by gavage with the principle component, DMT, quantified (250, 500 and 1000 μg mL^{-1}) or saline (sal). The control (CTRL) groups were given saline or *Ayahuasca*, also in three different doses. Thirty minutes after drug injection, the animals underwent the sexual behavior test. Following completion of the test, all animals were euthanized for blood collection.

2.5. Reagents and chemicals

Sodium borohydride ($NaBH_4$) sodium borate, HPLC-grade methanol, acetonitrile, triethylamine and phosphoric acid were purchased from Merck (Darmstadt, Germany). Tryptamine, harmine hydrochloride and harmaline hydrochloride were

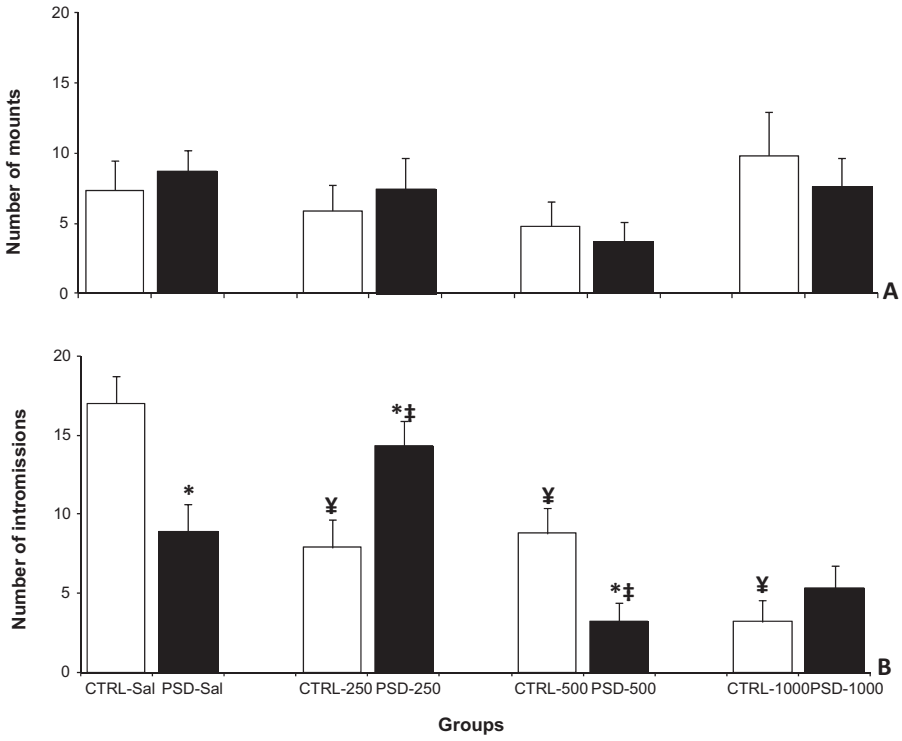

Fig. 3. The number of mounts (panel A) and intromissions (panel B) in the PSD saline (sal) or *Ayahuasca* (dose) groups compared with the respective CTRL group ($n = 10$/groups). * Different from respective control rats, ¥ different from CTRL-sal and ‡ different from PSD-sal.

purchased from Sigma Co. (St Louis, MO, USA). Ultra-pure deionized water was supplied by a Milli-Q RG unit from Millipore (Bedford, MA, USA).

2.6. Ayahuasca extract

The *Ayahuasca* extract was obtained from stocks which the police had seized, since it is not legal in Brazil to use the substance except in a religious context. The DMT (404 μg mL), harmine (451 μg mL), harmaline (124 μg mL) and tetrahydroharmine (1482 μg mL), active constituents found in *Ayahuasca*, were determined by high performance liquid chromatography with diode array detection (HPLC-DAD) and conducted by the Criminalistics Institute of São Paulo.

2.7. Synthesis of tetrahydroharmine

The synthesis of tetrahydroharmine (THH) was performed according to the procedure previous published (Callaway et al., 1996). In summary, harmaline hydrochloride was slowly added to a methanolic sodium borohydride solution at 0 °C. After 40 min, the reaction mixture was acidified with HCl, alkalinized with NaOH and extracted with dichloromethane. The organic layer was dried with magnesium sulfate, filtered and evaporated under vacuum. The evaporated residue was twice recrystallized with ethanol, generating off-white crystals with a melting point of 197 °C. The structure was confirmed on the basis of the 1H NMR, GC–MS and ESI–MS.

2.8. Synthesis of dimethyltryptamine

The synthesis of dimethyltryptamine (DMT) was performed according to a modified procedure based on the selective dimethylation method described previously (Pires et al., 2009; Giumanini et al., 1980). In summary, sodium borohydride was slowly added to a stirred solution of tryptamine in tetrahydrofuran at 0 °C. Afterwards, sulfuric acid and an aqueous solution of formaldehyde were also added to the reaction mixture. This solution was diluted with water and alkalinized with NaOH pellets until pH 14 was reached. The obtained product was extracted with diethyl ether. The organic layer was dried with magnesium sulphate, filtered and evaporated under vacuum. The product was purified by means of a silica chromatographic column (eluted with *n*-hexane-ethyl acetate 80:20) and recrystallized with *n*-hexane-ethyl acetate (80:20). White crystals with a melting point of 64 °C were obtained with this procedure. The structure was confirmed on the basis of the 1H NMR, GC–MS and ESI–MS.

2.9. Blood sampling and hormone determination

Immediately after behavioral testing, all rats from the CTRL and PSD groups were taken to an adjacent room and decapitated. Blood samples were collected and stored individually. Blood was collected in glass tubes and centrifuged at 3018.4 × *g* for 15 min at room temperature and then frozen at −20 °C until assayed. Serum testosterone [Intra-assay coefficient of variation (ICV = 7.7%) and progesterone (ICV = 6.5%) were measured by a chemiluminescent enzyme immunoassay (Advia Centaur, Bayer Corporation, USA). Plasma corticosterone (ICV = 7.1%) levels were assayed using a double antibody radioimmunoassay method specific for rats and mice using a commercial kit (MP Biomedicals, USA). The sensitivity of the assay was 0.25 ng mL^{-1}.

2.10. Statistical methods

The results of the sexual behavior tests did not meet assumptions of normality and thus were evaluated statistically with the Kruskal–Wallis non-parametric test (all groups: CTRL and PSD and different doses) followed by the Mann–Whitney test (comparing group by group). Regarding the hormonal concentrations, the groups were compared by ANOVA followed by the Tukey's

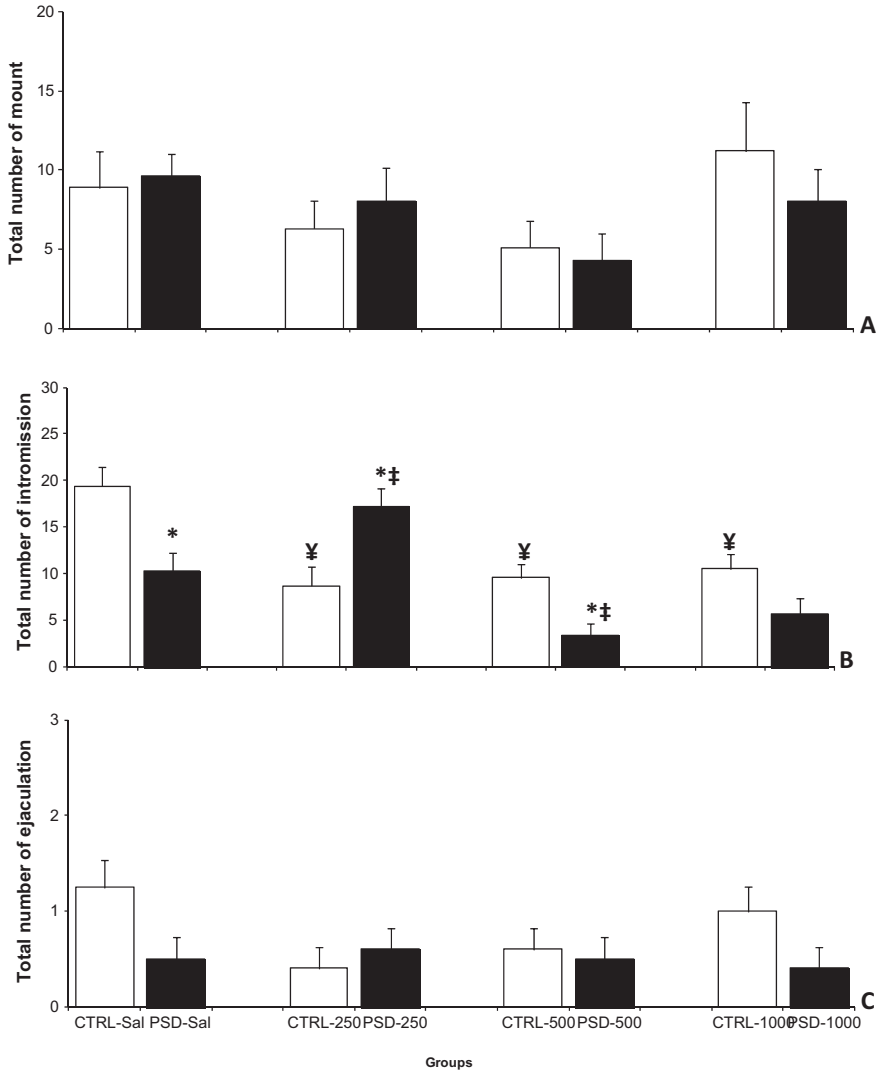

Fig. 4. Effect of combined *Ayahuasca* intake and paradoxical sleep deprivation (PSD) on total number of mounts (panel A), intromissions (panel B), and ejaculations (panel C) ($n = 10$/groups). * Different from respective control rats, ¥ different from CTRL-sal and ‡ different from PSD-sal.

post-hoc test. The values are expressed as means ± standard error of the mean (SEM). The level of significance was set at $p < 0.05$.

3. Results

3.1. Sexual behavior parameters

The effect of the *Ayahuasca* in rats that had normal sleep showed a significant reduction in the number of intromissions (preceding the first ejaculation) and a total number of intromission at all three doses in CTRL rats in relation to CTRL-sal ($p < 0.002$, $p < 0.004$, and $p < 0.006$, Figs. 3B and 4B). PSD-sal rats had a lower number of intromissions and total number of intromissions compared to the CTRL-sal ($p < 0.02$, Figs. 3B and 4B). In addition, PSD rats that received 500 μg mL^{-1} of *Ayahuasca* presented a significant reduction in number of intromissions and total number of intromissions relative to the respective CTRL and PSD-sal groups ($p < 0.01$ and $p < 0.006$, Figs. 3B and 4B). The lower dose in PSD-250 μg mL^{-1} produced a significant increase in the number and total number of intromissions compared to respective CTRL and PSD-sal groups ($p < 0.01$ and $p < 0.02$, Figs. 3B and 4B). No significant differences were observed for latencies of mount, intromission and ejaculation (Fig. 2) and numbers of mounts and ejaculations (Figs. 3 and 4A and C) parameters among the groups.

3.2. Hormone concentrations

3.2.1. Testosterone

The ANOVA followed by Tukey's post-hoc test revealed a significant decrease (45%) in testosterone concentration in PSD-sal compared to the CTRL-sal group ($p < 0.05$, Fig. 5A). In addition, a trend toward decreased testosterone in PSD-500 ng mL^{-1} rats were observed in comparison with PSD-sal ($p < 0.07$). There were no other significant differences.

3.2.2. Progesterone

Progesterone concentrations were similar in all groups, except for PSD-500 μg mL^{-1} rats, which presented lower levels compared with the respective CTRL-500 μg mL^{-1} group ($p < 0.03$; Fig. 5B).

3.2.3. Corticosterone

Fig. 5C shows the effects of PSD and *Ayahuasca* on corticosterone levels. The ANOVA revealed no significant difference among the groups ($p > 0.05$).

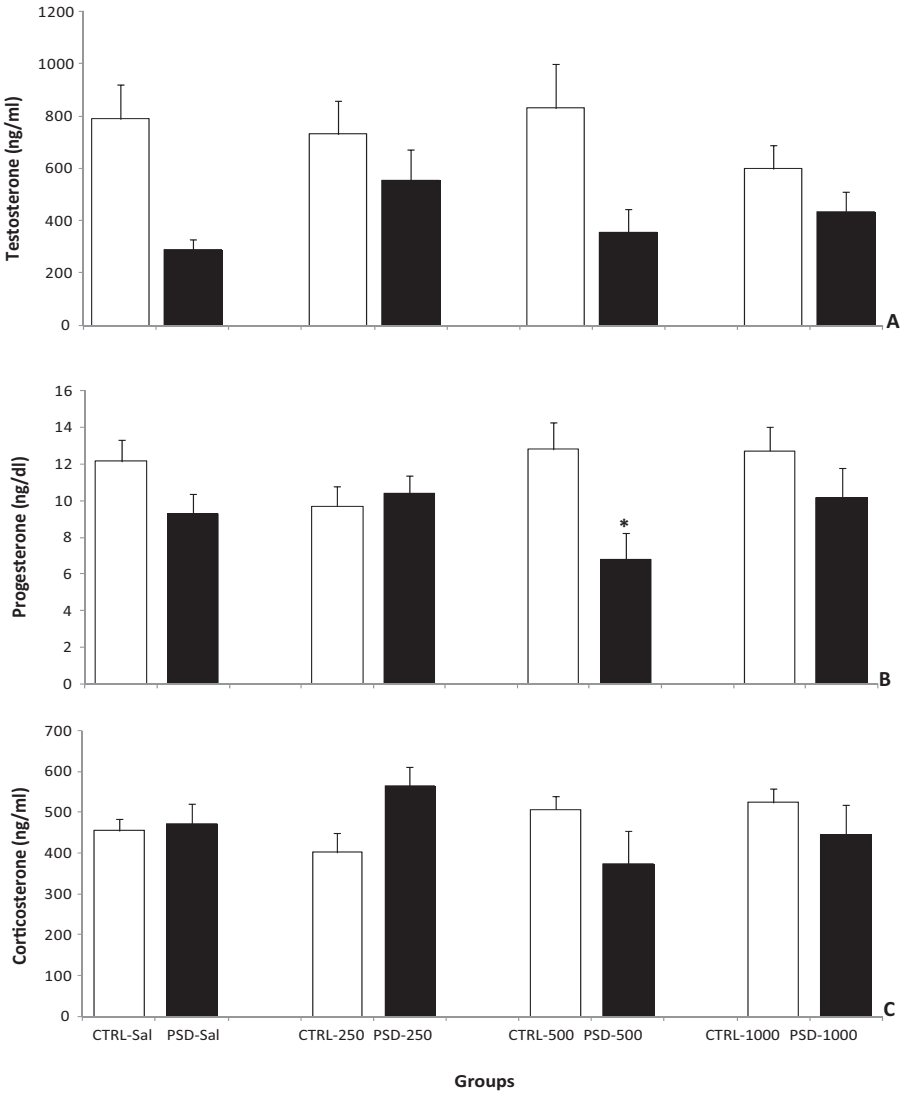

Fig. 5. Mean ± SEM concentrations of serum testosterone (in ng mL^{-1}, panel A), progesterone (in ng mL^{-1}, panel B) and corticosterone (in ng mL^{-1}, panel C) in saline/*Ayahuasca* control (CTRL) and paradoxical sleep deprivation (PSD) male rats (n = 10/groups). * Different from respective control rats.

4. Discussion

This study was designed to examine the effects of *Ayahuasca* either alone or in combination with PSD on the sexual response of male rats. *Ayahuasca* intake alone markedly impaired sexual performance, while the association with sleep deprivation had significant, but heterogeneous effects on male sexual performance.

The practice of drinking *Ayahuasca* has migrated to the United States and Europe (Ott, 1999; Santos et al., 2007) where it is consumed by members of religious sects and by casual users. Currently, it is well documented that *Ayahuasca* can alter different behaviors. Emotional, perceptual, and memory changes have been reported in humans following an average dose (150 mL) (Keiser et al., 2009; McKenna, 2004; Shanon, 2003). Our data showed that sexual performance, reflected by number of intromission and total number of intromission, was decreased by all doses of *Ayahuasca* in control rats with normal sleep pattern when compared with the respective control saline group.

Previously, it has been shown that sexual behavior can be modulated by the lack of sleep (Alvarenga et al., 2009; Andersen and Tufik, 2008; Andersen et al., 2011). Our data support these findings. For instance, the number and total number of intromissions in the PSD-sal group were significantly lower compared to the CTRL-sal group. As expected, 96 h of PSD hampered sexual behavior in male rats and this result was accompanied by a decrease in testosterone concentration. Moreover, the effects of sleep deprivation interacted with the effects of the *Ayahuasca* drug.

With respect to the testosterone profile, our findings demonstrated a significant reduction in this in PSD-sal rats and a trend toward decreases ($p < 0.07$) in PSD-500 ng mL^{-1} rats. Testosterone is a hormone particularly affected by sleep deprivation in rats. A series of studies have demonstrated the drastic effects that PSD has on androgen levels. Decreased concentrations of testosterone in sleep deprived male rats have been consistently observed (Andersen et al., 2004, 2005; Alvarenga et al., 2009). Since testosterone is involved with male sexual function, this response was related to a low sexual performance, corroborating the influence of *Ayahuasca* intake on male sexual response modulated by testosterone. In our data, we did not find a good association between the hormonal and sexual response. However, little is known about the hormonal changes resulting from the ingestion of *Ayahuasca* and even more when combined with PSD.

On the other hand, when *Ayhuasca* was combined with PSD, the lower dose induced an increase of sexual performance whilst the

intermediate dose produced a great impairment of sexual performance compared with the non-PSD CTRL group at the same dose. Corroborating our previous findings this is probably due to the effect of progesterone on male sexual behavior (Andersen et al., 2004, 2007; Alvarenga et al., 2009, 2010; Andersen and Tufik, 2005). Previously, it has been demonstrated that male rats with sexual experience with good sexual performance have higher levels of progesterone (Alvarenga et al., 2010). Accordingly, the rats with low performance should present a decrease of progesterone concentration. In the present study, we observed that only the group that was sleep-deprived and given 500 ng mL^{-1} of *Ayahuasca* presented both low performance and reduced progesterone concentration. The absence of alteration of progesterone in the other PSD groups could be attributed to a high progesterone concentration in these subjects (promoted by sexual experience) prior to PSD protocols. In this sense, only the group that received intermediate dose had lower concentrations of progesterone and decrease in sexual behavior.

Until now, no study has investigated the effect of *Ayahuasca* alone or in combination with PSD on sexual behavior. In general, *Ayahuasca*, independent of the dose, produced important alterations in sexual performance in both control and sleep-deprived rats. Although this change has been observed in sexual response, the complete mechanisms of *Ayahuasca* are not totally clear; thus, it is possible that other physiological and central pathways besides those discussed here are involved. In conclusion, the current data demonstrate that *Ayahuasca*, which is commonly used in religious cults, can modulate sexual response.

Author contributions

TAA was responsible for conception, writing, and is the leading author of the article. TAA, DNP, GM and VAG were responsible for conduct of animal experimentation. JLC was responsible for supplied *Ayahuasca* solution with active principles in defined concentrations. MLA and ST were responsible for editing assistance and drafting of the critical revision for important intellectual content. All authors give final approval of the version to be published. Also, the authors declare that they have no conflicts of interest.

Competing interests

The authors are not part of any associations or commercial relationships that might represent conflicts of interest in the writing of this study.

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